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RESEARCH ARTICLE

Pharmacognostical, Phytochemical and Anti-bacterial Studies of a *Siddha* Medicine: *Gunmakudori* (*Persicaria hydropiper* (L.) Delarbre) Leaves

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Abstract

Background: Medicines and drug precursors have been developed from medicinal plants, which have played a decisive role in global therapeutic and veterinary applications. Siddha and Ayurvedic medicine have traditionally used the *Persicaria hydropiper* plant for its traditional applications for gastric ailments. Even though it is praised in folklore, scientific investigations into its activities still need to be completed. **Aim:** Various medicinal properties of the plant, including antimicrobial, antidepressant, hepatoprotective, anti-oxidant, cardioprotective, anti-diabetic, and anti-cancer properties, can be found in its coumarin, steroids, and anthraquinones. **Methods:** This study meticulously evaluated *P. hydropiper* leaves for their physicochemical properties, phytochemical screening, elemental analysis, chemical profiling, and antimicrobial and antibacterial activities. **Results:** In the methanol extract, 21.8mg of GAE, 23.3mg of QE, and 6.663 mg of catechin equivalents were found in phenol, flavonoid, and condensed tannin. Under UV at 254nm and 366nm, HPTLC fingerprinting profiles reveal seven spots with different Rf values, indicating a unique chemical signature. At various concentrations, Ca, Fe, O, N, and other elements were detected by XRF analysis. In the disc diffusion method, methanol and water extracts show significant anti-bacterial effects on *K. pneumoniae, P. aeruginosa,* and *S. aureus*. It was found in GC-MS that the methanol extract contained several compounds that demonstrated gastroprotection activity in several studies, which may be related to the traditional medical claims of the plant for dyspepsia and stomach pain caused by ulcers. **Conclusion:** Furthermore, this research sheds light on the varied pharmacological activities of *P. hydropiper* and its intricate pharmacognostic details. Standardization of herbal drugs is urgently needed, supporting traditional claims through scientific validation. These derived reports can be used to develop pharmacopoeial monographs. *P. hydropiper* can now be optimally utilised in modern medicine with these insights for researchers, pharmacologists, and practitioners.

Major Findings: The plant, *P. hydropiper* was found rich in polyphenols and flavonoids, with various concentrations of Ca, Fe, O, and N elements. GCMS analysis showed fifty-nine active principles in which major compounds are biologically gastroprotective. The extracts showed antibacterial activity on *K. pneumoniae, P. aeruginosa,* and *S. aureus.*

Keywords: HPTLC, Gastroprotection, GC-MS, *Gunmakudori, Gunmam, Persicaria*

1. Introduction

Herbal remedies have played a vital role in the prevention and treatment of diseases, with applications in both human and veterinary medicine. As a reliable source of medicinal compounds and precursors for synthetic drugs, herbs have been extensively documented in traditional medicine systems, including *Ayurveda*

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and *Siddha* texts[1]. Despite the availability of various traditional herbal therapies and extensive research, the lack of scientific communication has led to the exclusive preservation of conventional knowledge without proper scientific documentation[2]. Due to the increasing demand for herbs, there is an urgent need to enhance research in pharmacognosy to provide authentic and detailed information to a broader medical area[3]. *P. hydropiper* (L.) Delarbre, also synonymous with *Polygonum hydropiper*, is a widely available plant native to countries with tropical-temperate climates, such as India, China, Japan, and Bangladesh. It grows up to 2 feet in height and is known by various names in different regions. The herb has traditionally been used in *Ayurvedic* and *Siddha* medicine to treat infertility, dyspepsia, haemorrhoids, and diarrhoea. Primarily it is used in *Siddha* medicine for treating throbbing pain (*soolai*) and gastric ulcer (*gunmam*) as given in the texts, *Gunapadam mooligai*[4]. It is known as *Bishkatali* in Bengali and scientific investigations are yet to be done on its folklore applications, like its potential against snake and insect bites[5-7]. Folklore medicine has documented the plant as a remedy for headaches, toothaches, gastric ulcers, and liver enlargement, among other applications[8]. Tribes in Arunachal Pradesh have used the leaf paste as fish poison[9]. Also, the paste of the plant's roots is applied on wounds and carbuncles, which heals them quickly[10]. The plant contains phytoconstituents like coumarins, steroids, anthraquinones, terpenoids, flavonoids, and phenolics, which contribute to its antimicrobial, antidepressant, hepatoprotective, anti-oxidant, cardioprotective, anti-diabetic, and anti-cancer potential[5,7].

Despite the high demand for herbal drugs, concerns about their quality frequently arise because of quality control investigations and label claims. Standardizing phytochemical, pharmacological, and analytical screening is imperative in establishing scientific evidence for traditional claims[11]. The WHO recommends macroscopic, microscopic, and physico-chemical examination and phytochemical evaluation to determine the standard of medicinal plants[12]. There needs to be more literature on the pharmacognostic profile of *P. hydropiper* despite extensive discussions of its pharmacological profiles. This study evaluates the plant's physicochemical properties, extractive values, and phytochemical evaluation to report standardization parameters. Additionally, the disc diffusion method was used to test the antimicrobial properties of the plant against Klebsiella, Staphylococcus, and Pseudomonas bacteria. Vancomycin was used as the standard. Due to the lack of published data supporting gastric activity and standardization of the drug, these parameters help prepare monographs to assess the drug's quality and support the potential therapeutic benefits of the plant for gastro-intestinal disorders as described in the following verse from *Agathiyar Gunapadam* a *Siddha* text.

"Cūlaiyō ṭiṉai tolaiyākkā caṅkayanōy
kālaṉeṉṟa kuṉmaṅ kalaṅkuṅkāṉ - ñālamicai
naṉmaik kuriyakuṇa nalkuvameṉ ṟāṟṟiluṟuṅ
kuṉmak kuṭōritaṉakku"

The study evaluates the medicinal properties of *Gunmakudori* (*P. hydropiper* (L.) Delarbre) in *Siddha* medicine. It includes assessing organoleptic properties, conducting preliminary phytochemical analysis, estimating phytoconstituents, and studying the anti-bacterial activity of methanol and aqueous extracts. The aim is to explore the plant's potential therapeutic benefits for gastrointestinal disorders.

2. Methodology

2.1 Collection and Authentication of Plant

Persicaria hydropiper was procured from the riverside of the Saththiyar Dam, Gengamuthoor, Madurai, Tamil Nadu, in May. It was duly authentified by Dr. D. Aravind, Assistant Professor in Medicinal Botany, National Institute of Siddha, Chennai. The herbarium sample was preserved for future reference.

2.2 Organoleptic Evaluation

Fresh leaves are collected and checked for colour, odour, and taste by sensory characters.

2.3 Pharmaceutical Analysis of Powder

The powder was subjected to bulk and tapped density, and a 1% solution was checked for pH using a pH meter.

2.4 Determination of Physico-chemical Parameters

Plant aerial parts were thoroughly washed and dried at ambient temperature for five days under shade[13]. The plant parts are ground into powder and passed through

sieve 40. Loss on drying (LOD; moisture contents), pH, ash values, and extractive values using various solvents were measured, and fluorescence analysis was conducted[14]. The heavy metal content was determined using standard procedures[15].

2.5 Microscopic Analysis of Leaves and Stem

The parts were cleaned and shade-dried to remove moisture from aerial plant parts. The cleaned parts were dipped in formalin for fixation and were cast into blocks using dyes of paraffin[16]. Thin slices were sliced using a rotary microtome and coloured using a safranin stain[17]. The stained sections were placed under a microscope fitted with a Nikon lab Photo 2 camera with a binocular lens. Description of the anatomical features were marked and described as per standard books[18].

2.6 Plant Extraction

The powdered visible plant parts of *P. hydropiper* were extracted successively using pet-ether, chloroform, ethyl-acetate, methanol, and ethanol, as well as with double distilled water using a Soxhlet apparatus[19]. The extracts were collected and filtered through the Whatman No. 1 filter paper. Filtrates were reduced with a rotary evaporator, and their solid mass was noted.

2.7 Preliminary Phytochemical Screening

The dry extracts were tested for various chemical constituents such as carbohydrates, proteins, glycosides, alkaloids, phenols, steroids, flavonoids, tannins, volatile oils, fats, saponins, *etc.*[20,21].

2.8 Total Phenolic Content Estimation (TPC)

The total Phenol content of the methanol extract was performed using the standard procedure of Folin Ciocalteu reagent[22]. 1ml of methanol extract (100µg/ml) was added with Folin Ciocalteu reagent (2.5ml; 10%w/v). This solution was mixed with a sodium carbonate solution (2ml; 75%) and allowed to settle for 5 min. The solution was allowed to react for 10min at 50°C with continuous stirring. The solution was subjected to UV to measure absorbance at 765nm. The standard curve of Gallic acid was constructed with various concentrations (50, 100, 150, 200, and 250 µg/ml) using a similar method, and the amount of TPC in the extract was counted as milligrams of Gallic Acid Equivalents (GAE)/g of dry extract.

2.9 Total Flavonoid Content Estimation (TFC)

The flavonoidal content of the methanol extract was calculated using Dowd's method[23]. The standard drug (Quercetin) was dissolved in distilled water (25, 50, 100, 150, and 200 µg/mL) and mixed with $AlCl_3$ solution (2ml; 10%w/v) with methanol and potassium acetate solution (0.2ml; 1M). The reaction mixture was incubated at 35°C for 30-45min and subjected to absorbance measurement at 415nm. The standard curve of quercetin was drawn and replaced with methanol extract (100µg/mL) to measure TFC in quercetin equivalents as mgQE/g of extract.

2.10 Total Tannin Content Estimation

The total condensed tannin content was calculated using the standard method with minor modifications. To the methanol extract (50µl of 10%w/v in methanol), vanillin solution (3ml of 4%w/v in methanol) was mixed with 1.5ml of conc. HCl. This reaction mixture was incubated for 15 min and recorded for absorbance at 500nm against methanol. The TTC was expressed as mg of catechin equivalents/g of extract[24].

2.11 HPTLC Fingerprinting Analysis

A 2.5gm extract of *P. hydropiper* was solubilised in 100ml of HPTLC-grade methanol and subsequently set for centrifugation at 1000rpm for 5 min. The resulting solution was filtered through micropore filter paper, and a 2µL aliquot of the above solution was utilised for sampling. The HPTLC analysis was conducted on precoated silica gel $60F_{254}$ TLC plates measuring 7x10cm. The plates were sampled with the extract solution at a 5mm band width, employing a CAMAG Linomat-5 applicator with Hamilton's syringe. The plates were developed by mobile phase Chloroform- ethyl-acetate-formic acid (5-4-1) to 90mm. The designed plates were dried to evaporate the solvents, and normal light and a CAMAG 4 visualiser equipped with winCATS software were used for visualization at 254 and 366nm. Bands and peaks were carefully observed, recorded, and tabulated for further analysis[25-28].

2.12 XRF Analysis

To analyze the multi-elemental compositional analysis of plant materials, a Si/Li semiconductor detector, with a resolution of 200e, 5.9keV 55Fe, featuring a 0.25mm Be-Window and a 12mm diameter, was employed. This detector was coupled with a multichannel analyzer EG and G ORTEC, utilizing calibration sources 238Pu, 109Cd, 125I, and 241Am. The readings were conducted with geometrical and non-coaxial sources and sample detector arrangement. Dried *P. hydropiper* powder, achieved by drying at 105°C, was used, followed by grinding and homogenization through a 40-mesh sieve. The pellets were prepared using a hydraulic press (20mm diameter, 300mg) at 10MPa. To introduce a known amount of elements, a standard solution containing 100.0 µg Fe, 10.0 µg Cu, 15.0 µg Zn, 5.0 µg Pb, and 10.0 µg Se was deposited onto a pellet using a micropipette. The pellet was dried at room temperature, homogenized, and made into pellets under similar conditions. The signals of analysis for the poly elemental standards were seen by calculating the count's difference between the standard and the powder and pellets[29].

2.13 GCMS Study

This study used Thermo GC-Trace Ultra (5) and Thermo MS-DSQ (II) equipment. The analysis utilised an anon-polar DB (35) MS column with Capillary Standard, measuring 30mmx0.25mmx0.25µm film. A flow rate of helium was set at 1ml/min. The determination of constituents was dependent on compound libraries by Willey and NIST[30], incorporating retention indices integrated into the GC-MS instrument for comparison[31,32].

2.14 Microbial Cultures and Media

Mueller-Hinton agar media and nutrient broth were purchased from Sigma Aldrich India Ltd. Isolated pure cultures of *Klebsiella pneumonia, Staphylococcus aureus*, and *Pseudomonas auregenosa* were procured from the Microbiology lab of Apollo Hospitals, Chennai, and identified and isolated further in the lab[33]. The obtained cultures were incubated in the nutrient broth overnight at 35ºC to prepare subcultures. The bacterial cultures were harvested with saline solution, and the cell counts were maintained at 10^7 CFU/ml by reading the absorbance at 580 using a spectrophotometer[34].

2.15 Investigation of *In Vitro* Anti-bacterial Activity

Due to the better yields and a more diverse range of phytoconstituents observed in the preliminary results of the phytochemical evaluation, the methanol extract was chosen for further investigation. In contrast, the ethanol and aqueous extracts were combined to form hydro-alcoholic extracts to investigate anti-bacterial activity through the disc diffusion method. To prepare standard stock solutions, extracts were dissolved in distilled water to achieve a final concentration of 100mg/ml. This solution was sterilised, filtrated, and stored in Eppendorf tubes at 4°C until use. Sterile blank discs (6mm diameter) were loaded with the extract and standard drug (Vancomycin 30µg/ml) solution at a concentration of 10mg/disc and stored overnight at -5°C[35,36] For the anti-bacterial assay, 1ml of bacterial culture was diluted to 10^5 CFU/ml using a saline solution, and 15ml of this solution was used to inoculate freshly poured sterile Mueller-Hinton agar media (10ml) in a petri dish. Subsequently, filter paper loaded with the extract solution was placed on the culture-loaded Petri dishes, and the dishes were stored at 4°C to facilitate drug diffusion into the medium. The dishes were then incubated at 37°C for 24 h. A vernier caliper was used to measure all zones of inhibition[37,38].

3. Results

3.1 Organoleptic Evaluation

The leaves of *P. hydropiper* are dark green (Figure 1) with a characteristic odour and spicy taste.

3.2 Pharmaceutical Analysis of Powder

The dried powder is smooth and homogenous, with a bulk density of 0.2816g/ml and a tapped density of 0.3942g/ml. pH of 1% aqueous solution was found to be 5.77.

The aerial parts of the plant were observed under a microscope, and images in Figure 2 reveal the presence of chloride band cells, glandular trichomes, and spongy parenchyma consistent with structure standards in gymnosperms[39,40].



Figure 1.1 Habitat of *P. hydropiper*



Figure 1.2 Dorsal view of leaf



Figure 1.3 Ventral view of leaf

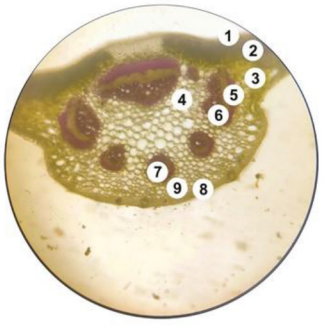
Figure 1. The habitat, dorsal, and ventral surface view of the leaf of *P. hydropiper*.

Table 1. Pharmaceutical analysis of *P. hydropiper* powder

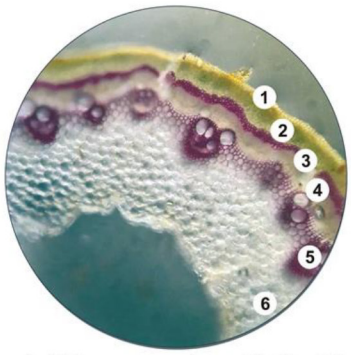
Parameters	Results
Morphology and texture	Green and smooth powder
Bulk density	0.2816g/ml
Taped density	0.3942g/ml
pH(1%w/v solution)	5.77

Table 2. Physiochemical Parameters of the aerial parts of *P. hydropiper*

S. No.	Parameters	Results (%w/w)
1	Foreign organic matter	Nil
2	LOD at 105°C	7.21
3	Total Ash	6.41
4	Acid-insoluble ash	0.1433
5	Aqueous extractive value (WSE)	15.8
6	Alcohol extractive value (ASE)	11.8
7	Petroleum ether Yield	5.44
8	Chloroform Yield	6.29
9	Ethyl acetate Yield	8.88
10	Methanol Yield	23.6
11	Ethanol Yield	11.19
12	Aqueous Yield	2.55



(1) Upper epidermis (2) Palisade (3) Spongy parenchyma
(4) Parenchyma (5) Sclereids (6) Xylem (7) Phloem
(8) Glandular Trichome (9) Collenchyma



(1) Epidermis (2) Spongy parenchyma (3) Sclereid band cells
(4) Parenchyma (5) Vascular bundle (6) Spongy parenchyma

Figure 2. Microscopy of *P. hydropiper* leaf and stem.

The Ash values of the aerial plant parts were estimated using standard procedures, which showed total ash of 6.41%w/w and acid insoluble ash of 0.1433%w/w. The LOD values of the powder were determined as 7.21%w/w, which was under limits (IP, 1996) (Table 1). The values of water and ethanol soluble extract were estimated as 15.8 and 11.8%w/w, respectively. The percentage yield of the extracts was found to be PEPH, CEPH, EEPH – MEPH, AEPH- 5.44%w/w,6.29%w/w, 8.88%w/w, 23.62%w/w, 11.19%w/w and 2.55%w/w (Table 2).

3.3 Phytochemical Screening of Extracts of *P. hydropiper*

The composition of chemical constituents in various extracts is tabulated in Table 3. Carbohydrates were

identified in methanol, ethanol, ethyl-acetate, and aqueous extracts, while flavonoids were identified in aqueous, methanol, ethanol, chloroform, and ethyl-acetate extracts. Alkaloids were not detected in any extract. Starch was absent in all extracts. Proteins were identified in methanol and water extracts. Sterols were observed in chloroform, petroleum ether, and also with ethyl acetate extract. Tannin compounds were identified in ethyl acetate, ethanol, and methanol extracts, while terpenoids were found in petroleum ether, chloroform, and ethyl-acetate extract. Mucilage and glycosides were not detected in any of the extracts.

3.4 Phenolic, Flavonoid and Condensed Tannin Content

The extract's TPC, TFC, and TTC were determined as 21.8mg GAE/g, 23.3mg QE/g, and 6.663mg catechin equivalents/g.

3.5 HPTLC Fingerprinting

HPTLC fingerprinting profile of extract of aerial parts of *P. hydropiper* shows seven spots at Rf values of 0.02, 0.23, 0.32, 0.42, 0.62, 0.68, 0.85 under UV at 254nm, as shown in Figure 3.

3.6 XRF Analysis of *P. hydropiper*

The XRF of the methanol extract of *P. hydropiper* reveals an overview of its elemental composition, presented in both oxide and primary forms in Table 4. The dominant elements in oxide form include Calcium Oxide (CaO) at 36.96%, Potassium Oxide (K_2O) at 21.32%, and Magnesium Oxide (MgO) at 13.25%. Additionally, significant proportions of Sulfur Trioxide (SO_3), Chlorine (Cl), and Phosphorus Pentoxide (P_2O_5) are detected at concentrations of 10.06%, 5.52%, and 5.52%, respectively. The elemental analysis highlights the prevalence of Oxygen (O) at 32.00%, Calcium (Ca) at 26.41%, and Potassium (K) at 17.70%. Noteworthy elements such as Silicon (Si), Sodium (Na), and Aluminum (Al) are also present in smaller amounts.

3.7 GC-MS Study

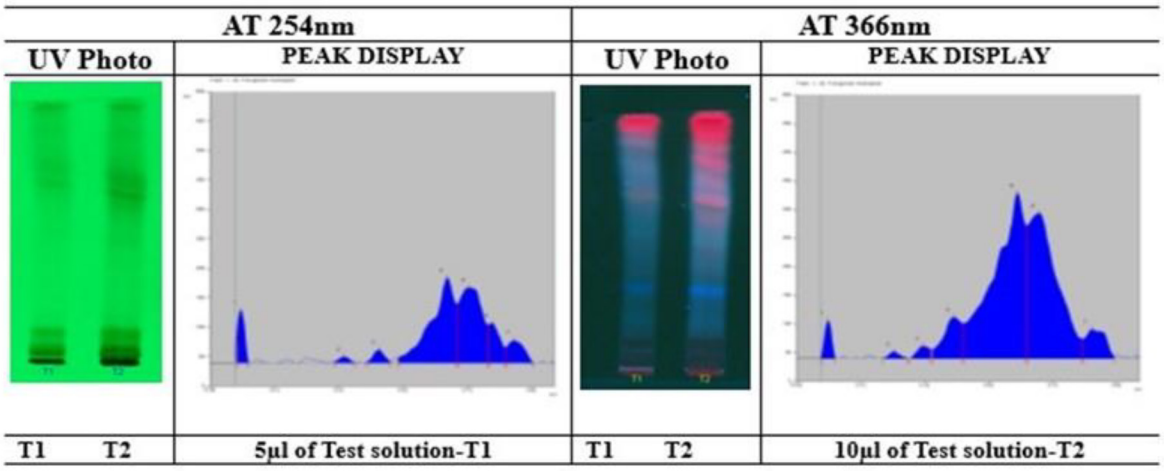
GCMS analyzed the plant extract for its bioactive chemical content. Fifty-nine active principles were tabulated in Table 5 and Figure 4.

3.8 Antimicrobial Activity of *P. hydropiper*

Table 6 illustrates the anti-bacterial properties of different extracts from *P. hydropiper* on three bacterial microorganisms, namely *Klebsiella, Pseudomonas,* and *Staphylococcus*. The *P. hydropiper* extract exhibited an inhibitory zone, measuring 15.11±1.42mm against *K. pneumoniae*, 15.21±2.01mm against *P. aeruginosa*, and 15.54±1.87mm against *S. aureus*. Similarly, the *P. hydropiper* aqueous extract showed inhibitory activity with mean diameter zones of 15.35±1.75mm, 15.32±2.11mm, and 15.44±1.77mm against *Klebsiella pneumonia, Pseudomonas aeruginosa* and *Staphylococcus aureus,* respectively as in Figure 5. These results suggest that the methanolic and water

Table 3. Preliminary phytochemistry analysis of *P. hydropiper*

S. No.	Parameters	Results					
		Pet. Ether	Chloroform	Ethyl-Acetate	Ethanol	Methanol	Water
1	Appearance	Yellow coloured mass	Brown coloured mass	Brown coloured mass	Brown coloured mass	Brown coloured mass	Brown coloured mass
2	Alkaloids	-	-	-	-	-	-
3	Carbohydrates	-	-	-	+	+	+
4	Flavonoids	-	+	+	+	+	+
5	Starch	-	-	-	-	-	-
6	Proteins	-	-	-	-	+	+
7	Sterols	+	+	+	-	-	-
8	Tannins	-	-	-	+	+	+
9	Terpenoids	+	+	+	-	-	-
10	Mucilage	-	-	-	-	-	-
11	Glycosides	-	-	-	-	-	-



Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	51.0	0.02	89.3	17.85	0.04	0.5	1394.1	6.92	unknown *
2	0.31	2.2	0.34	10.5	2.10	0.38	0.3	262.5	1.30	unknown *
3	0.41	1.1	0.45	22.5	4.50	0.48	3.1	584.6	2.90	unknown *
4	0.51	9.3	0.66	144.8	28.94	0.69	99.8	7881.3	39.14	unknown *
5	0.70	99.9	0.73	127.5	25.50	0.79	64.8	6724.5	33.39	unknown *
6	0.79	65.1	0.80	67.5	13.49	0.84	26.4	1832.0	9.10	unknown *
7	0.85	26.4	0.87	38.1	7.62	0.93	0.1	1457.3	7.24	unknown *

Seven spots at R_f values under UV at 254 nm were observed.

Figure 3. HPTLC of methanolic extract *P. hydropiper.*

Table 4. XRF analysis of methanol extract of *P. hydropiper*

Oxides of element		Elemental form	
Name	**Amount (%)**	**Name**	**Amount (%)**
CaO	36.96	O	32.00
K_2O	21.32	Ca	26.41
MgO	13.25	K	17.70
SO_3	10.06	Mg	7.99
Cl	5.52	Cl	5.52
P_2O_5	5.52	S	4.03
SiO_2	4.81	P	2.41
Na_2O	1.08	Si	2.25
Al_2O_3	1.07	Na	0.80
MnO	0.19	Al	0.57
Fe_2O_3	0.13	Mn	0.15
PbO	0.10	Fe	0.09
-	-	Pb	0.09

extracts from *P. hydropiper* exhibit anti-bacterial activity against the tested microorganisms.

4. Discussion

The plant of the present study, *P. hydropiper*, is valuable in treating gastric problems in *Siddha*, *Ayurveda* and folk medicine[4]. Literature reveals that despite the more significant therapeutic potential, the plant had been under-investigated for its phytochemical and pharmacological profile. Thus, this research was undertaken to establish a detailed study of various plant extracts' physic-chemical parameters and photochemistry. Addressing the standardization of the plant, the focus was given to the gastro-protective activity of the plant as supported by anti-inflammatory, anti-oxidant, and anti-bacterial effects[41].

The dry powder from the aerial parts of the plant contains 7.12%w/w moisture, which indicates that there is no excess moisture present. This is a significant

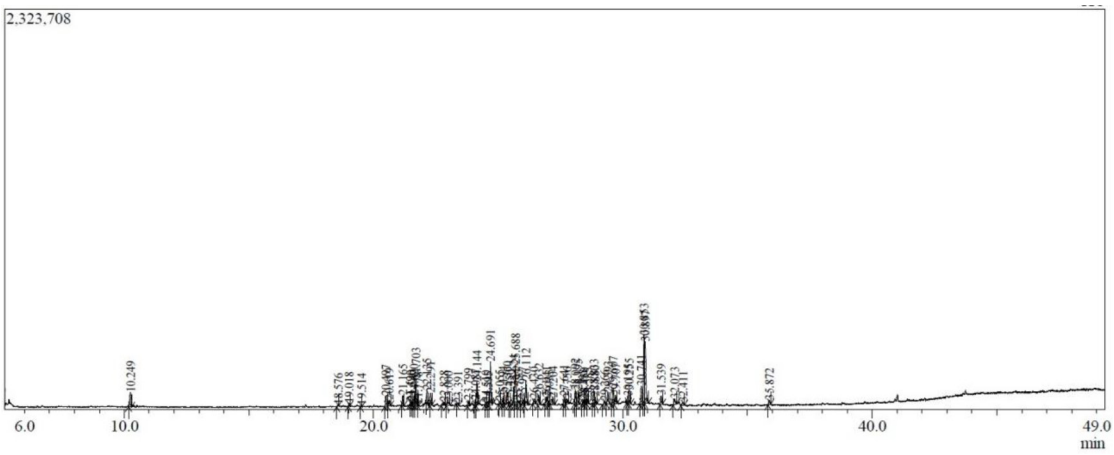


Figure 4. GCMS study of *P. hydropiper* extract.

Table 5. GC-MS investigation of extract of *P. hydropiper*

Peak#	T (min)	Name	Formula	Area%
1	10.249	Undecane	$C_{11}H_{24}$	2.42
2	18.576	Humulene	$C_{15}H_{24}$	0.15
3	19.018	1-(1,5-dimethyl-4-hexenyl)-4-methyl-Benzene	$C_{15}H_{22}$	0.37
4	19.514	3,5-bis(1,1 -dimethyl ethyl)-Phenol	$C_{14}H_{22}O$	0.14
5	20.497	2(1H)-Benzocyclooctenone, decahedron-10a-methyl-, trans-	$C_{13}H_{22}O$	1.87
6	20.619	1-methyl-2,4-bis(1-methyl-ethenyl)-Cyclo-Hexane	$C_{13}H_{22}$	1.04
7	21.165	Caryophyllene oxide	$C_{15}H_{24}O$	1.17
8	21.490	(-)-Isopinocampheol, Pentafluoropropionate	$C_{13}H_{17}F_5O_2$	0.15
9	21.540	Cycloisolongifolene	$C_{15}H_{24}$	0.27
10	21.620	2-Allylphenol	$C_9H_{10}O$	1.13
11	21.703	(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxa-bicyclo [9,1,0] dodeca-3,7diene	$C_{15}H_{24}O$	3.47
12	21.778	9.11(13)-Eremophiladien-12-oic acid, methyl ester	$C_{16}H_{24}O_2$	0.19
13	22.135	Humulenol-II	$C_{15}H_{24}O$	1.39
14	22.291	8-bromo-Neoisolongifolene	$C_{15}H_{23}Br$	1.48
15	22.828	14-Hydroxycaryophyllene	$C_{15}H_{24}O$	0.80
16	23.000	(2,4a,5,8a-tetra-methyl-1,2,3,4,4a,7,8,8a-octahedron-1-naphthalene)ester-Acetate	$C_{16}H_{26}O_2$	0.88
17	23.391	1,1a,4,5,6,7,7a,7b-octahedron-1,1,7,7a-tetramethyl-,(1aα.,7α.,7aα,7bα.)-2H-Cyclopropa[a]naphthalene-2-one	$C_{15}H_{22}O$	0.46
18	23.799	1-methyl-4-(2-methyloxiranyl)-7-Oxabicyclo[4.1.0]-heptanes	$C_{10}H_{16}O_2$	0.71
19	24.087	7-Hydroxy-6,9a,dimethyl-3-methylene-decahydro-azuleno[4,5b]furan-2,9-dione	$C_{15}H_{20}O_4$	0.73
20	24.144	Drim-7-en-11-ol	$C_{15}H_{26}O$	3.08
21	24.510	Loliolide	$C_{11}H_{16}O_3$	0.26
22	24.595	Z-(13,14-Epoxy) tetradec-11-en-1-ol-acetate	$C_{16}H_{28}O_3$	0.25
23	24.691	Drim-7-en-11-ol	$C_{15}H_{26}O$	6.07
24	25.055	1,2,3,6,7,8,8a,8b-octahedron-4,5-dimethyl-Biphenylene	$C_{14}H_{20}$	0.28
25	25.194	3-hydroxy-2-methyl-4-[4-t-butyl]-Butanal	$C_{15}H_{22}O_2$	0.78
26	25.360	2,4a,5,8a-tetramethyl-1,2,3,4,4a,7,8,8a-octahydronaphthalen-1-yl-formate (isomer-1)	$C_{15}H_{24}O_2$	1.73
27	25.450	Humulane-1,6-diene-3-ol	$C_{15}H_{26}O$	0.46
28	25.624	Caryophyllane,4,8-.beta.-epoxy	$C_{15}H_{26}O$	2.73

Table 5. Continued...

Peak#	T (min)	Name	Formula	Area%
29	25.688	Cyclohexene, 1,4,6,6-tetramethyl-	$C_{10}H_{18}$	7.16
30	25.793	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	1.23
31	25.977	3a,9-Dimethyl,dodeca-hydrocyclohepta[d]indene-3-one	$C_{16}H_{26}O$	1.04
32	26.112	Allyl-ionone-4	$C_{16}H_{24}O$	3.49
33	26.430	2-methyl-4-(2,6,6-trimethyl-1-cyclohexenyl)-But-3-enal	$C_{14}H_{22}O$	0.93
34	26.632	Xysmalogenin	$C_{23}H_{32}O_4$	1.41
35	26.941	[5-(Hydroxy-methyl)-2,5,8a-trimethyl-1,4,4a,6,7,8-hexa,hydro-naphthalene-1-yl]methanol	$C_{15}H_{26}O_2$	1.66
36	27.033	Corymbolone	$C_{15}H_{24}O_2$	0.47
37	27.204	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	0.82
38	27.641	9-cedranone	$C_{15}H_{24}O$	1.13
39	27.756	Octadecanoic acid	$C_{18}H_{36}O_2$	0.97
40	28.092	3a,4,5,6,6a,6b-hexahydro-5,5,6b-trimethyl-,(1a.alpha.,3a.beta.,6a.beta.,6b.alpha.)-(+)-Cycloprop[e]-indene-1a,2(1H)-carboxaldehyde	$C_{15}H_{20}O_2$	1.88
41	28.195	Bohlmann k2631	$C_{15}H_{20}O_2$	2.36
42	28.393	Tetradecahydro-4b,8,8-trimethyl-[3S-(3.alpha.,4a.beta.,4b.alpha.,8a.beta.,10a.alpha.)]-3-Phenanthrenol	$C_{17}H_{30}O$	0.56
43	28.450	Costunolide	$C_{15}H_{20}O_2$	0.61
44	28.585	14-Epiandrographolide	$C_{20}H_{30}O_5$	0.58
45	28.833	1,3,4,5,5a,6,7,8,9,9a-decahydro-6,6,9a-trimethyl-Benzo[e]isobenzofuran-1,4-dione	$C_{15}H_{20}O_3$	1.80
46	28.880	(5aα,9aα)-4,5,5a,6,7,8,9,9a-octahydro-6,6,9a-trimethylnaphthol[1,2-c]furan-(3H)-one (isodrimenin)	$C_{15}H_{22}O_2$	1.04
47	29.266	8,9-dehydro-9-formyl-Cycloisolongifolene	$C_{16}H_{22}O$	0.37
48	29.393	3a,4,5,6,6a,6b-hexahydro-5,5,6b-trimethyl-,(1a.alpha.,3a.beta.,6a.beta.,6b.alpha.)-(+)-Cycloprop[e]indene-1a,2(1H)-carboxaldehyde	$C_{15}H_{20}O_2$	2.07
49	29.607	Drim-7-en-11-ol	$C_{15}H_{26}O$	2.53
50	29.707	Costunolide	$C_{15}H_{20}O_2$	1.67
51	30.195	Phytol	$C_{20}H_{40}O$	0.99
52	30.255	(5aα.,9aβ.,9bβ.)-5,5a,6,7,8,9,9a,9b-octahydro-6,6,9a-trimethylnaphtho[1,2-c]furan-1-(3H)-one (dimension)	$C_{15}H_{22}O_2$	1.98
53	30.741	1,4,4a,5,6,7,8,8a-octahedron-2,5,5,8a-tetramethyl-1-Naphthalenemethanol	$C_{15}H_{26}O$	2.87
54	30.853	(1R-7S,E)-7-Isopropyl-4 and 10-dimethylenecyclodec-5-enol	$C_{15}H_{24}O$	10.21
55	30.897	[5-(Hydroxymethyl)-2,5,8a-trimethyl-1,4,4a,6,7,8-hexahydronaphthalene-1-yl]methanol	$C_{15}H_{26}O_2$	9.73
56	31.539	11-hydroxy-,(11α.)-Pregn-4-ene-3,20-dione	$C_{21}H_{30}O_3$	1.38
57	32.073	1,4,4a,5,6,7,8,8a-octahedron-2,5,5,8a-tetramethyl-1-Naphthalenemethanol	$C_{15}H_{26}O$	1.24
58	32.411	trans-Longipinocarveol	$C_{15}H_{24}O$	0.66
59	35.872	dodec-2-en-1-yl 2-ethyl phenyl ester-succinic acid	$C_{24}H_{36}O_4$	0.71

Table 6. Anti-bacterial activity zone of *P. hydropiper* extracts

Extracts	Bacterial microorganism's mean diameter inhibition zone (mm)		
	Klebsiella pneumoniae	***Pseudomonas aeruginosa***	***Staphylococcus aureus***
P. hydropiper methanolic extract	15.31±1.42	15.33±2.01	15.54±1.87
P. hydropiper water extract	15.15±1.75	15.12±2.11	15.24±1.77
Vancomycin (30µg/ml)	12.33±1.21	11.04±2.54	13.27±2.15

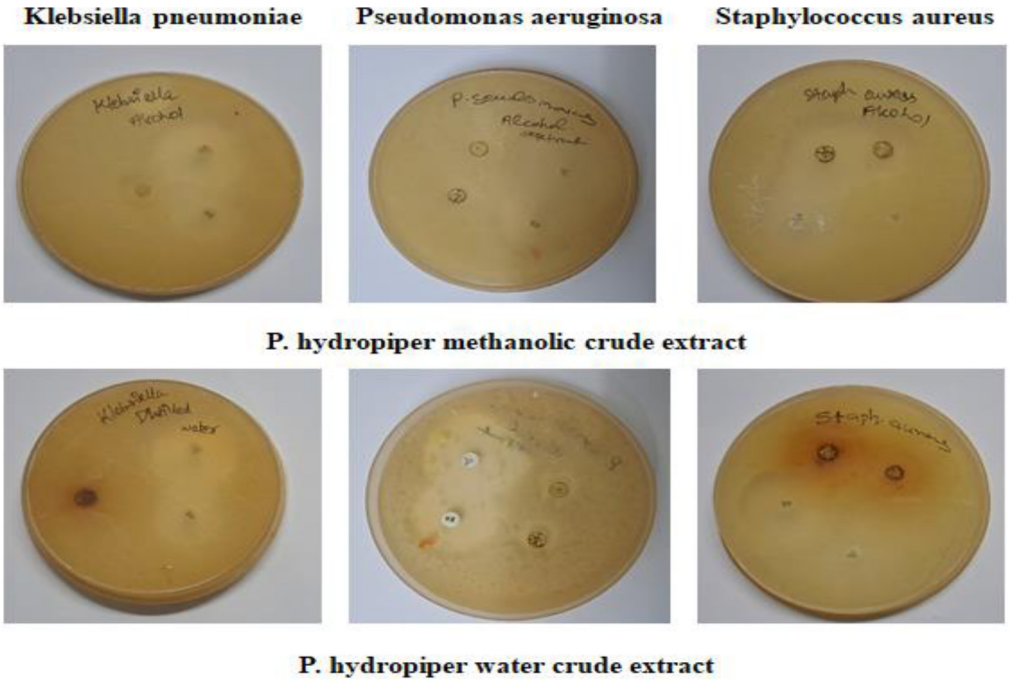


Figure 5. Anti-bacterial activity of *P. hydropiper* methanolic and aqueous extracts.

step in controlling the growth of microorganisms in the powdered drug sample. As the moisture content is below the par allowed moisture content of 10-12%, it can be advocated that the powered sample was dried properly[42]. Ash values of the powdered sample were also established, indicating the drug's identity. Extractive values of the drug suggest that the plant contains more polar constituents that range from phenols, flavonoids, carbohydrates, proteins, and other polar soluble components. Successive extraction of the sample also supported the claims of the presence of polar soluble constituents in the drug.

The methanol extract was found to contain flavonoids, sterols, tannins, terpenoids, and carbohydrates with a yield above 20%, indicating that methanol extract had the highest amount of chemical constituents followed by ethanol extract. Since ethanol and water are similar in polarity, both extracts were combined and considered as hydro-alcoholic extracts in further investigations.

The anti-bacterial activity of the extracts showed results that were not different in both extracts. Overall, both extracts were active and potent against *Klebsiella*, *Pseudomonas,* and *Staphylococcus* bacteria. The results are consistent with previous studies that showed the anti-bacterial properties of the ethanol extract on *E. coli* and *Salmonella*[43]. The existence of several constituents earlier might be responsible for the activity against the bacteria above. As a part of the phytochemical investigation, TPC, TFC, and TTC were determined, and the results supported the existence of phenolic, flavonoidal, and tannin compounds consistent with the results of preliminary phytochemical screening. Quantifying these phytoconstituents also opens the scope for elucidation using advanced techniques like HPTLC.

HPTLC fingerprinting analysis revealed the presence of flavonoids in the mobile phase. Chloroform: Ethyl Acetate: formic acid elucidates flavonoids and polyphenols as per literature[44]. The R_f values of standard quercetin and rutin in the same mobile phase were determined as 0.852 and 0.66, corresponding to peak 7 at 0.87 and peak 4 at 0.66 in the methanol extract. The amounts of rutin and quercetin in the sampled aliquot were determined as 19.57µg and 3.62µg; therefore, the amount of both the compounds in methanol extract was calculated as 391.4µg/mg of rutin and 72.4µg/mg of quercetin which is known to exhibit significant protection against gastric ulcer and degeneration[45,46]. Supporting these claims, GC-MS analysis revealed the presence of 59 compounds, with the majority of compounds having gastro-protective

activity or other activities related to gastric, like anti-inflammatory effect in IBD, and anti-oxidant activity, which is a common mechanism in most of the gastro-protective drugs, especially of herbal origin.

Compound undecane has been investigated to have antimicrobial activity[47]. Caryophylline is proven to possess gastro-protective activity against NSAID-induced gastric degeneration and inhibiting helicobacter infection, which is common in gastric problems[48,49]. (1R, 3E, 7E, 11R)-1,5,5,8-Tetra methyl-12-oxabicyclo[9.1.0]dodeca-3,7diene is a stereoisomer of humulene epoxide known to inhibit drug-metabolizing microsomal enzymes in the liver and exhibits anti-inflammatory activity and anti-oxidant activity especially to treat IBD[50.51].

8-bromo-Neoisolongifolene isolated from *Lippia citrodora* was attributed to anti-oxidant and antimicrobial properties[52]. Xysmalogenin, which contributed to about 1.5% of the methanol extract, was a significant constituent in *Xysmalobium undulatum*, which was used to treat stomach cramps and was proven to exhibit antidiarrhoeal activity[53]. Drimane sesquiterpenoids like Drim-7-en-11-ol were found to be about 3% in the extract was established to show antifungal activity against *C. albicans*[54,55] and exhibited anti-bacterial properties on pulmonary and skin disorders[56]. The primary ingredient in methanol extract, costunolide, inhibited gastric ulceration induced by ethanol[57,58]. The plant *P. hydropiper* is proven to cure various diseases as per literature[4-7] Especially; the infusion of the plant was used to treat colic pain due to gastro-intestinal disorders[4,59,60].

From the isolated phytoconstituents, squalene, tetradecanoic acid, n-hexadecanoic acid, and piperine were proven to show anti-oxidant, 5-α-reductase inhibition, hemolysis, anti-fibrinogen, and antimicrobial properties[61,62]. A phenol-based compound, squalene, found in the resins and latex of various plants, was found to show defence against different disease-causing organisms and assist chemotherapy against colon cancer[63,64]. Along with anti-cancer, it also was proven to show gastroprotective and hepatoprotective activities, pesticidal and sunscreen properties[65,66]. Various health benefits of piperine were documented in the literature[67].

Water and ethanol extracts have a higher number of phytochemicals. Water extract exhibits significant Antimicrobial activity around the 15mm zone of inhibition. Methanol has high extractive value. HPTLC showed 7 effective compounds in the extract. XRF offers elemental compositions similar to antacids containing calcium, magnesium, and aluminium. In GCMS, among 59 compounds, 25 have specific actions on the gastrointestinal tract. Many compounds have anti-neoplastic and analgesic actions. Octadecadienoic acid inhibits inflammation, lowers cholesterol, and reduces arthritic pain[68], and Naphthalene was found to have good anti-bacterial properties[69].

Isopinocampheol is a primary compound found in *H. officinalis* and has been proven to have antifungal activity. It prevents gastric ulcers caused by ethanol and oxidative stress[70]. Cycloisolongifolene isolated from the volatile oil of *Curcuma* species exhibited anti-oxidant cytotoxic properties against LNCaP and BS6 cell lines[71]. Volatile oils from *Cyperus* species contain sesquiterpenes like corybolone, which showed antiplasmodial activity, and the attributions of the gastric protection against damage induced by ethanol in mice were given to the volatile oils in the plant[72]. Trans-longipinocarveol is a compound isolated from the volatile oil of *Ocimum* species, which was proven to show anti-inflammatory activity[73] and antiulcer, analgesic, and anthelmintic activities[74]. *P. hydropiper* was known to treat dyspepsia and haemorrhoids in *Siddha*, *Ayurveda* and folk medicine is supported by the presence of the above chemical constituents with gastro-protective properties and anti-inflammatory activity[5], also supporting claims of the use of the plant for functional dyspepsia in Iranian folklore[75]. The plant's analgesic components are responsible for using *P. hydropiper* to relieve labour pains[76,77].

5. Conclusion

The main aim of the investigation was to standardize the plant extracts and to explore the potential therapeutic benefits of the plant as a natural remedy for gastrointestinal disorders, as mentioned in *Siddha* medicine. In conclusion, this study comprehensively explores *P. hydropiper*, shedding light on its rich phytochemical profile and pharmacological potential. The plant's traditional uses in *Siddha*, *Ayurveda* and Folk medicine find scientific support by identifying various bioactive

compounds, affirming its application in gastric problems. The derived reports of pharmacognostic and phytochemical investigation of the leaf can be used as a reference and included in pharmacopoeial monographs to draw the standards for this plant. The antibacterial activity demonstrated by methanol and water extracts against *K. pneumoniae*, *P. aeruginosa*, and *S. aureus* underscores the therapeutic potential of *P. hydropiper* in combating bacterial infections. A deeper investigation into the specific mechanisms underlying the observed pharmacological activities could provide insights into the plant's mode of action. Further studies could delve into quantifying and characterizing phytochemical compounds, facilitating the development of gastric-targeted pharmaceutical formulations. Collaborative efforts between traditional *Siddha* practitioners and allopathic scientists could bridge the gap between traditional knowledge and scientific documentation, strengthening a holistic approach to herbal medicine. Moreover, the integration of advanced analytical techniques and computational methods could enhance our understanding of the complex interactions within *P. hydropiper*. In conclusion, the multifaceted nature of *P. hydropiper* invites diverse research opportunities, ranging from elucidating its molecular mechanisms to refining cultivation practices. As the scientific community continues to unveil the secrets of this medicinal herb, the potential for innovative therapeutic solutions and sustainable utilization remains promising.

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7. Author Contribution

Jeyavenkatesh: Conception and design, Writing, Field visit, Collection; Ramkumar: Visualization, Oversight, Leadership; Savariraj Sahayam: Analysis, Interpretation; Krishnaveni: Research data management; Roja Ramani: Study materials, Data acquisition. All authors have read and agreed to the published version of the manuscript.

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